# UltraScence Atto Western Substrate

**01 DEC 2022**

| Catalog Number | Size | Reaction(s) |
|---|---|---|
| CCH385-B100ML | 50 ml x 2 | Sufficient for over 25 mini-gel size membranes. |
| CCH385-004ML | 2 ml x 2 | Sufficient for over one mini-gel size membrane. |

## Storage Conditions

Stable for up to 24 months at 4°C.

## Description

The UltraScence Atto Western Substrate, as a luminol-based enhanced chemiluminescent substrate, is the most sensitive and brightest ECL Western Substrate among our UltraScence product lines for low-femtogram to high-attogram detection of antigen with excellent sensitivity and long signal duration.

UltraScence Atto Western Substrate is compatible with conducting immunoblots with horseradish peroxidase (HRP) – conjugated secondary antibodies. Further, its long chemiluminescent signal duration makes both digital and film-based imaging possible without any loss of the signal.

## Kit Content(s)

| Catalog Number | Size |
|---|---|
| CCH385-B050MLA | 50 ml x 1 |
| CCH385-B050MLB | 50 ml x 1 |

## Required materials but not provided

- A compatible Chemiluminescence or X-ray Imaging Systems
- A plastic sheet protector or plastic wrap to prevent the membrane from drying

## Instrument Compatibility

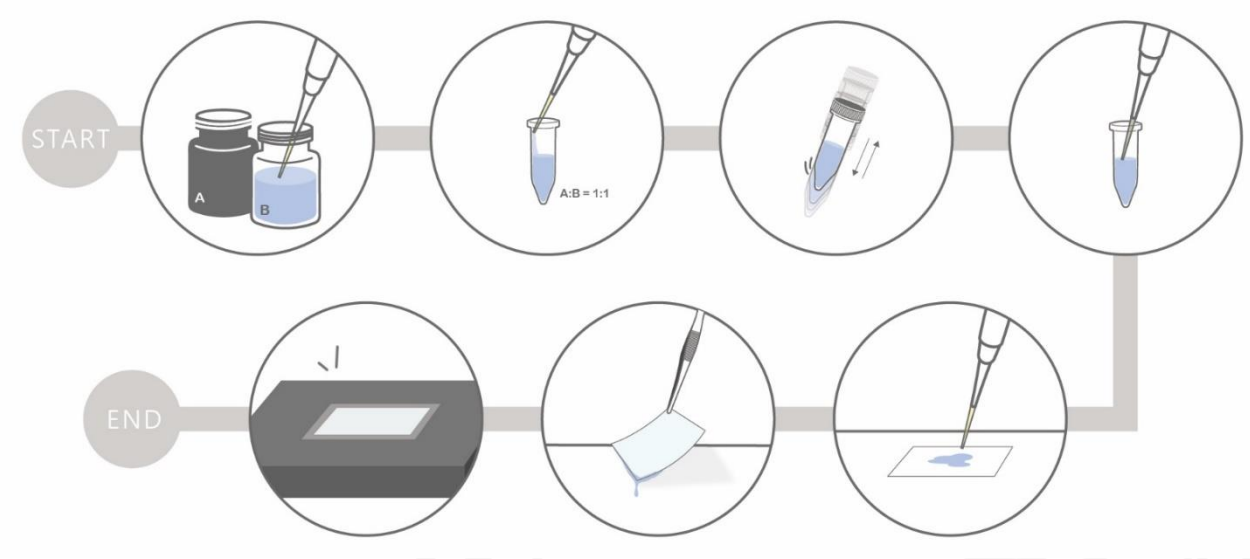
This western substrate is compatible with the majority of commercially available Chemiluminescence and X-ray Imaging Systems.

## Reaction Setup

1. Keep the membrane moist in the wash buffer while preparing the substrate mixture. Please ensure the membrane does not dry out during the subsequent steps.
2. Mix Luminol solution and Peroxide Solution in a 1:1 ratio, and thoroughly agitate the chemiluminescent substrate solution well for preparing the 0.1 ml of solution / $cm^2$ of membrane.

- For a mini-sized membrane (7 x 8.5 cm), 4 ml of solution is sufficient.
- For a midi-sized membrane (8.5 x 13.5 cm), 10 ml of solution is sufficient.

3. Place the membrane with the protein side up on a clear and level surface or in a clean container.
4. Remove the membrane from the chemiluminescent substrate solution and drain off excessive solution.
5. Place the membrane in a plastic sheet protector or in plastic wrap to prevent the membrane from drying.
6. Image the membrane with a digital imager or by exposing to the X-ray film.




## Important notes

UltraScence ECL substrates series is compatible with the use from low picogram to low-femtogram level detections. Please kindly refer to the ECL selection guide of UltraScence Western substrate as the below table.

| Bio-Helix Western Substrates | Advantages for you | Sensitivity | Compare Performance to |
|---|---|---|---|
| UltraScence Pico Plus Western Substrate CCH321-B100ML | **Best value** of abundant protein detection and best sensitivity among entry-level western substrate | low picogram or high femtogram | -Thermo Scientific™ Pierce ECL Substrate<br>-Thermo Scientific™ SuperSignal™ West Pico PLUS<br>-CYANAGEN WESTAR NOVA 2.0<br>-Advansta WesternBright™ ECL<br>-Abcam High Sensitivity ECL Substrate Kit |
| UltraScence Pico Ultra Western Substrate CCH345-B100ML | **Better choice** when seeking low abundance proteins, over **30 times** sensitivity than UltraScence Pico Plus western substrate. | low picogram to mid femtogram | -Millipore™ Immobilon™ Western Substrate<br>-FUJIFILM Wako ImmunoStar Zeta<br>-Cytiva Amersham ECL Prime<br>-Advansta™ WesternBright™ Quantum™<br>-Abcam Very High Sensitivity ECL Substrate Kit<br>-CYANAGEN WESTAR ETA C ULTRA 2.0<br>-Thermo Scientific™ SuperSignal™ West DURA |
| UltraScence Femto Plus Western Substrate CCH375-B100ML | **Born to seek**, seeking less abundance proteins in your Western Blot, even low femtograms. | mid femtogram to low femtogram | -FUJIFILM Wako ImmunoStar LD<br>-GeneTex Trident femto<br>-Thermo Scientific™ SuperSignal™ West Femto<br>-Advansta™ WesternBright™ Sirius™<br>-Abcam Ultra High Sensitivity<br>-CYANAGEN WESTAR SUPERNOVA<br>-Cytiva Amersham™ ECL Select™ |
| UltraScence Atto Western Substrate CCH385-B100ML | **Break the record**, providing the most sensitive and brightest protein signal for your Western Blot. | Low femtogram to high attogram | -CYANAGEN WESTAR HYPERNOVA<br>-Thermo Scientific™ SuperSignal™ West Atto |

## Troubleshooting

| Problem | Cause | Solution |
|---|---|---|
| High Background | Overconcentrated primary or secondary antibody | *Decrease the antibody concentration. |
| | | *Perform a dot blot to optimize the concentration. |
| | Insufficient wash | *Increase the frequency or duration. |
| | Incomplete blocking | *Decrease the antibody concentration. |
| | | *Perform a dot blot to optimize the concentration. |
| No Reaction or Weak Signal | Insufficient antigen binding | *Decrease antibody concentration.<br>*Optimize blocking reagents for achieving a balance between sensitivity and specificity. |
| | Poor antibody binding to the antigen | *Optimize detergent used for antibodies.<br>*Increase the antibody incubation time. |
| No Reaction or Weak Signal | Proteins washed from the membrane during assay | *Reduce the number or intensity of wash |
| | Insufficient reagent volume | *Apply additional volumes of antibody blocking reagent, or wash solution. |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